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(54) Title: METHODS OF PREPARATION OF LIVE ATTENUATED BACTERIAL VACCINES BY ALTERATION OF DNA ADENINE METHYLASE (DAM) ACTIVITY IN THOSE BACTERIA

(57) Abstract: Live attenuated bacteria vaccines are provided. Also provided are methods by which such vaccines can be obtained, including: a method by which a copy of the *dam* gene from a pathogenic bacteria is cloned into a plasmid capable of replication in the same bacteria species such that it is overexpressed from either a *lac* promoter, *tac* promoter, *araBAD* promoter, *trc* promoter, *trp* promoter, T7, SP6, or T5 bacteriophage promoters, a native promoter from that species, or other appropriate promoter. The plasmid containing the *dam* gene is then transferred into the pathogens to cause increased expression of Dam resulting in the formation of a live attenuated bacterial vaccines. Alternative methods for producing the vaccine are also provided including altering or replacing the chromosomal promoter for the native *dam* gene so as to alter Dam expression or mutating or replacing the native *dam* gene so as to alter the expression of Dam in a pathogenic bacteria.

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/00740

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A01N 63/00; A61K 48/00; C12N 1/20

US CL : 424/93.2, 93.4, 93.42; 435/252.3

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/93.2, 93.4, 93.42; 435/252.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2002/0068068 A1 (MAHAN et al) 6 June 2002 (06.06.2002), paragraph 0046 and claims.	1-85

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

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- "O" document referring to an oral disclosure, use, exhibition or other means
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Continuation of B. FIELDS SEARCHED Item 3:
Medline, Biosis, Ca, Caplus, Embase, USPatfull
terms: bacteria, Dam, DNA, adenine methylase, alter?, mut?